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# GENETIC PREDICTORS OF HYPERGLYCEMIA DUE TO HYDROCHLOROTHIAZIDE THERAPY

Jorge L. Del Aguila

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GENETIC PREDICTORS OF HYPERGLYCEMIA DUE TO  
HYDROCHLOROTHIAZIDE THERAPY

by

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GENETIC PREDICTORS OF HYPERGLYCEMIA DUE TO  
HYDROCHLOROTHIAZIDE THERAPY

A  
THESIS

Presented to the Faculty of  
The University of Texas  
Health Science Center at Houston  
and  
The University of Texas  
M. D. Anderson Cancer Center  
Graduate School of Biomedical Sciences  
in Partial Fulfillment  
of the Requirements  
for the Degree of  
MASTER OF SCIENCE

By

Jorge L Del-Aguila, B.S., MS  
Houston, TX

May, 2012

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Finally, I would like to express my gratitude and thank to my parents, Norka and Luis Alberto, my brothers and to Sandra for her eternal friendship

# GENETIC PREDICTORS OF HYPERGLYCEMIA DUE TO HYDROCHLOROTHIAZIDE THERAPY

Publication No. \_\_\_\_\_

Jorge L Del Aguila, B.S., M.S.

Advisor: Eric Boerwinkle, Ph.D.

Response to pharmacological treatment is variable among individuals. Some patients respond favorably to a drug while others develop adverse reactions. Early investigations showed evidence of variation in genes that code for drug receptors, drug transporters, and drug metabolizing enzymes; and pharmacogenetics appeared as the science that studies the relationship between drug response and genetic variation.

Thiazide diuretics are the recommended first-line monotherapy for hypertension (i.e. SBP>140 or DBP>90). Even so, diuretics are associated with adverse metabolic side effects, such as hyperglycemia, which increase the risk of developing type 2 diabetes. Published approaches testing variation in candidate genes (e.g. the renin-angiotensin-aldosterone system (RAAS) and salt-sensitivity genes) have met with only limited success.

We conducted the first genome wide association study to identify genes influencing hyperglycemia as an adverse effect of thiazide diuretics in non-Hispanic White hypertensive patients participating in the Genetic Epidemiology of Responses to Antihypertensives (GERA) and Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) clinical trials. No SNP reached the *a priori* defined threshold of statistical significance ( $p < 5 \times 10^{-8}$ ). We detected 50 SNPs in 9 genomic regions with suggestive p-values ( $p < 1 \times 10^{-5}$ ). Two of them, rs6870564 (p-value =  $3.28 \times 10^{-6}$ ) and rs7702121 (p-value =  $5.09 \times 10^{-6}$ ), were located

close to biologic candidate genes, MYO and MGAT1, and one SNP in a genomic region in chromosome 6, rs7762018 (p-value= $4.59 \times 10^{-6}$ ) has been previously related to Insulin-Dependent Diabetes Mellitus (IDDM8).

I conclude that 1) there are unlikely to be common SNPs with large effects on the adverse metabolic effects to hydrochlorothiazide treatment and 2) larger sample sizes are needed for pharmacogenetic studies of inter-individual variation in response to commonly prescribed medication.

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## ABBREVIATIONS

2BHTR2B	5-Hydroxytryptamine (serotonin) Receptor
ADRB2	$\beta$ -2 adrenergic receptor
ALKBH8	Alkylation Repair Homolog 8 (E. coli)
AME	Adverse Metabolic Effects
BMI	Body Mass Index
CWF19L2	CWF19-like 2, Cell Cycle ontrol
DAG	Diacylglycerol
ELMOD1	ELMO/CED-12 Domain Containing 1
FTMT	Ferritin Mitochondrial
G1CCNG1	Cyclin
GERA	Genetic Epidemiology of Responses to Antihypertensives
GUCY1A2	Guanylate Cyclase 1, Soluble, Alpha 2
HCTZ	Hydrochlorothiazide
IDDM8	Insulin-Dependent Diabetes Mellitus
KCNJ1	Potassium Inwardly Rectifying Channel
LOX	Lysyl Oxidase
MAT2B	Methionine Adenosyltransferase II, Beta
MGAT1	Mannosyl(alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase
MYO10	Myosin X
NMUR1	Neuromedin U Receptor 1
NUDCD2	NudC Domain Containing 2

PEAR	Pharmacogenomic Evaluation of Antihypertensive Responses
PKC	Protein Kinases C
RAAS	Renin-Angiotensin-Aldosterone system
RHAMM	Hyaluronan-Mediated Motility Receptor
SCLT1	Sodium Channel and Clathrin Linker 1
SLC39A11	Solute Carrier Family 39 (metal ion transporter)- Member 11
SNCAIP	Synuclein- Alpha Interacting Protein
SRFBP1	Serum Response Factor Binding Protein 1
T1D	Type 1 Diabetes.
T2D	Type 2 Diabetes
ZNF474	Zinc Finger Protein 474

## Introduction

Hypertension is a major risk factor for stroke, cardiovascular and kidney diseases<sup>1</sup>. Hydrochlorothiazide (HCTZ) is a widely used diuretic recommended for part of the initial treatment of hypertension<sup>2</sup>. It is also one of the first drugs documented to improve clinical outcomes in patients with hypertension. However its ability to over-stimulate the RAAS (renin-angiotensin-aldosterone system) and to produce a variety of Adverse Metabolic Effects (AME) is well-known. These AMEs can be classified into two main types: electrolyte defects (hypokalemia<sup>3-7</sup>, hyponatremia<sup>8-10</sup>, and hyperuricemia<sup>11, 12</sup>) and metabolic abnormalities (hyperglycemia<sup>3, 13-15</sup> and dyslipidemia<sup>15-18</sup>). Among the metabolic abnormalities, hyperglycemia is particularly important because of the possibility of leading to a diagnosis of diabetes. A meta-analysis of 22 different clinical trials including 143,153 patients<sup>19</sup> showed that the odds ratio for incident of diabetes in antihypertensive clinical trials for HCTZ was 1.3. Although the risk is clinically significant, it is not observed in all patients. None the less, some clinicians suggest that the prescription of HCTZ should be avoided because of this adverse outcome<sup>20, 21</sup>

The precise mechanism by which HZCT influences glucose and triglyceride levels is not well understood. Three possible mechanisms have been proposed. The first possible mechanism suggests that inhibition of the renal sodium chloride channel by HZCT increases sodium and potassium excretion in urine<sup>16</sup>. The second states that the RAAS pathway is activated due to volume depletion, leading to increased aldosterone and potassium secretion<sup>16</sup>. The hypotheses conclude that hypokalemia induces higher proinsulin over

insulin secretion. Proinsulin is less active than insulin leading to an increased concentration of blood glucose<sup>2</sup>. The third possible mechanism suggests that increases in hepatic fat content decreases insulin sensitivity which produces hyperglycemia and most patients taking HCTZ develop higher hepatic fat content<sup>22</sup>.

One way to distinguish among these competing hypotheses, is to identify genetic variations that are predictive of inter-individual variation in the AME of HCTZ treatment. In previous targeted candidate gene studies, two loci have been reported to predict thiazide-induced hyperglycemia<sup>23</sup>: the potassium inwardly rectifying channel (KCNJ1) and the  $\beta$ -2 adrenergic receptor (ADRB2). However, no replication of these findings has been reported. An alternative approach to a candidate gene study is to query every gene and genomic region using a genome-wide association approach. In this thesis, I seek to identify genetic associations of HCTZ-induced change in plasma glucose that are replicated among two independent clinical trials in non-Hispanic whites, the Genetic Epidemiology of Responses to Antihypertensives (GERA) and Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR).

## **Materials and Methods.**

### *1. Study population*

The phenotype and genotype data were collected from The Genetic Epidemiology of Responses to Antihypertensives (GERA) study and The Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study. Both studies have been described previously <sup>24, 25</sup>.

In the GERA study, African-Americans and non-Hispanic Whites with essential hypertension were recruited at Emory University in Atlanta, GA, and at the Mayo Clinic in Rochester, MN, respectively. Essential hypertension was defined as blood pressure levels greater than 140/90 mmHg in the absence of a single known cause, or current use of prescription antihypertensive medications. Subjects with the following characteristics were not enrolled in the GERA study: allergy to hydrochlorothiazide; inability to discontinue antihypertensive medication, non-steroidal anti-inflammatory medication (aspirin > 325mg/d), liver disease, renal disease, diabetes mellitus (FBG > 140mg/dl or use of hypoglycemic medications), or use of oral contraceptives. The institutional review boards of the Mayo Clinic and Emory University approved the GERA study. The protocol for the GERA study was as follows: a wash-out period of at least 4 weeks was done in order to remove the effects of previous blood pressure medication. If during the wash out period an individual's blood pressure was > 180/110 mmHg, they were withdrawn from the study for safety reasons; if at the end of the wash-out period the diastolic blood pressure (DBP) was < 90mmHg, they were withdrawn from the study. Blood pressure was measured and blood samples were obtained for baseline biochemical measurements from the remaining

individuals. Qualifying individuals were given hydrochlorothiazide (25mg orally once daily) for 4 weeks. Subjects were asked to keep dietary sodium intake at approximately 2mmol/kg/day. A 24 hour urine sodium excretion measurement and food recall diaries were checked for dietary compliance. At the end of the 4 week diuretic treatment period, blood pressure was measured and blood samples were again obtained for biochemical measurements. In order to measure blood pressure at baseline and after diuretic treatment, individuals were in the seated position for 5 minutes before the reading was done by a trained nurse using a mercury sphygmomanometer. All blood collections were in the morning after 8 hours of fasting. For this project, we defined response to hydrochlorothiazide as the difference between the measurements collected after the 4 weeks of diuretic treatment and the baseline measurement.

In the PEAR study, any race-ethnicity gender combination from age 17 to 65 years old with mild to moderate essential hypertension was recruited. Subjects were enrolled from Gainesville, FL, Atlanta, GA, and Rochester, MN. All participants were newly diagnosed hypertensive, untreated hypertensive or treated hypertensive with less than three antihypertensive drugs. The exclusion criteria were: secondary hypertension, known cardiovascular disease, diabetes mellitus (type I or II) or FBG > 126mg/dL, primary renal disease, the presence of Raynaud syndrome (constriction of the small arteries in fingers and toes due to cold weather or emotional state), pregnancy or lactation, abnormal liver enzymes (AST, ALT or alkaline phosphatase > 2.5 times the upper limit of normal). The protocol of the study was as follows: a wash out period of at least two weeks was observed in order to remove the effects of previous blood pressure medication from the participants. A period of two weeks was used because in the GERA study the study investigator observed that stable

blood pressure rises were already obtained by this time. If at the end of the wash out period the average seated home DBP > 85mmHg and the home systolic blood pressure (SBP) < 180mmHg the individuals were enrolled into the randomized phase. All biological samples (blood and urine) were collected in the fasting state. As soon as the baseline studies were completed, the individuals were randomized to hydrochlorothiazide (diuretic drug-12.5mg orally once daily) or atenolol ( $\beta$ -blocker drug-50mg orally once daily). For this thesis research, I used only the patients randomized to the diuretic drug-arm from the PEAR study referred hereafter as PEAR monotherapy. If after 3 weeks the individual's average home and clinic blood pressure was > 120/70mmHg, then the dose of hydrochlorothiazide was increased (25mg orally once daily). All participants were examined in clinic every 10-21 days throughout the study.

For the purpose of this thesis research only non-Hispanic whites from GERA and PEAR were included.

## *2. Phenotype and Genotype data*

In the GERA study, plasma glucose concentrations were determined by automated spectrophotometric methods implemented on an IL Monarch Chemistry system 760 (Instrumentation Laboratories, Lexington, MA, USA). In the PEAR study, these methods were implemented on a Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN, USA). In both studies, plasma glucose concentrations were measured at the end of the drug-free period (baseline) and at the end of the hydrochlorothiazide period (final). Glucose response to hydrochlorothiazide was defined as the difference between the levels at the final and the baseline visits.



In the GERA study, a subset of individuals was genotyped using the GeneChip Human Mapping 500K Array, (Affymetrics, Santa Clara California, USA). The subset was selected based on the upper and lower tertile of DBP response. First, blood pressure measurements were adjusted for age and baseline DBP levels. Then, cases were defined as individuals from each race gender subgroup who had a DBP in the highest tertile and controls were defined as individuals from each race gender subgroup, who had a DBP in the lowest tertile. Each group had equal numbers of African American and non-Hispanics Whites.

PEAR participants were genotyped for the Illumina HumanOmni1-Quad (Illumina, San Diego, California, USA) using standard procedures. As part of routine quality control, SNP with minor allele frequency (MAF) <1%, call rates <95%, Hardy-Weinberg equilibrium p-values  $\geq 10^{-5}$  and individuals with more than 10% missing genotype were removed from the analysis.

### *3. Statistical analysis*

The software MACH<sup>26</sup> ([www.sph.umich.edu/csg/abecasis/MACH/download](http://www.sph.umich.edu/csg/abecasis/MACH/download)) was used for imputating the approximate 2.5 million HapMap SNPs using the reference panel for the European-Americans the Phase II CEU panel. Imputation results were filtered if the squared correlation between imputed and true genotypes (RSQ\_HAT) were below the threshold of 0.3 and  $MAF \leq 0.05$ . MACH generated a file with the highest posterior probabilities (i.e.dosage) at each imputed SNP which was used in the analysis.

To avoid the possibility of spurious association as the result of population substructure<sup>27</sup>,<sup>28</sup>, Principal Component Scores (PCS) were used as covariates in the analysis. These PCS were obtained using the EIGENSTRAT<sup>28</sup> software ([genepath.med.harvard.edu/~reich/EIGENSTRAT.htm](http://genepath.med.harvard.edu/~reich/EIGENSTRAT.htm)).

Linear regression was used to evaluate the association between each SNP and glucose response to hydrochlorothiazide. The variables sex, age, BMI, baseline glucose, and the first two principal component scores were used as covariates. A one degree of freedom additive model was applied. The analysis was performed using ProbABEL<sup>29</sup>. These genome-wide analyses were done separately for each study, and the results were subsequently meta-analyzed using a fixed effect model<sup>30</sup> with the inverse of the variance of the study-specific  $\beta$ -estimates to weight the contribution of each study. Meta-analysis was done using METAL ([www.sph.umich.edu/csg/abecasis/metal](http://www.sph.umich.edu/csg/abecasis/metal)). For the genome-wide analysis and the meta-analysis, the definition of statistical significance was taken to be  $p < 5 \times 10^{-8}$  and the direction of effect ( $\beta$ ) had to be the same in both studies. This Bonferroni-like correction reduces the probability of type I errors (false positives) but increases the probability of type II errors (false negatives). A statistically suggestive p value was defined as  $p < 1 \times 10^{-5}$ , and was used in order to avoid the rejection of possible true positive associations.

## Results

Characteristics of the study participants at baseline and after treatment are presented in Table 1A and Table 1B. The numbers of eligible participants in GERA and PEAR monotherapy were 196 and 229, respectively. In both studies, the percentage of male participants was higher than female participants (GERA 57.1% and PEAR 59.83%). The average age of the participants was almost the same in both studies (GERA 48.5 years and PEAR 49.97 years), as well as the average BMI values (GERA 31.3 and PEAR 30.3). In both studies, SBP was reduced by approximately 10mmHg, and DBP by approximately 5mmHg in response to HCTZ treatment. In the GERA study, these average blood pressure responses should not be over-interpreted because of the design of the genotyping sub study (see methods). The average plasma glucose and plasma cholesterol levels increased significantly after treatment with HCTZ in both studies ( $P<.0001$ ). The average triglyceride response was more pronounced in the GERA study compared to the PEAR study.

Variables	GERA: Non-Hispanic Whites		p
	baseline	Post-treatment	
Total	196		
Gender(% men)	57.1		
Age(y)	48.5±7.3		
Body mass index(BMI)	31.3±5.6		
Plasma Glucose(mg/dL)	92.4±12.5	95.1±13.2	<.0001
Total Cholesterol(mg/dL)	187.8±31.2	194.85±34.1	<.0001
Plasma triglyceride(mg/dL)	184.3±97.6	213.98±164.45	<.0001
Systolic blood pressure(mmHg)	142.6±12.6	131.7±13.5	<.0001
Diastolic blood pressure(mmHg)	95±5.7	89.4±9.2	<.0001

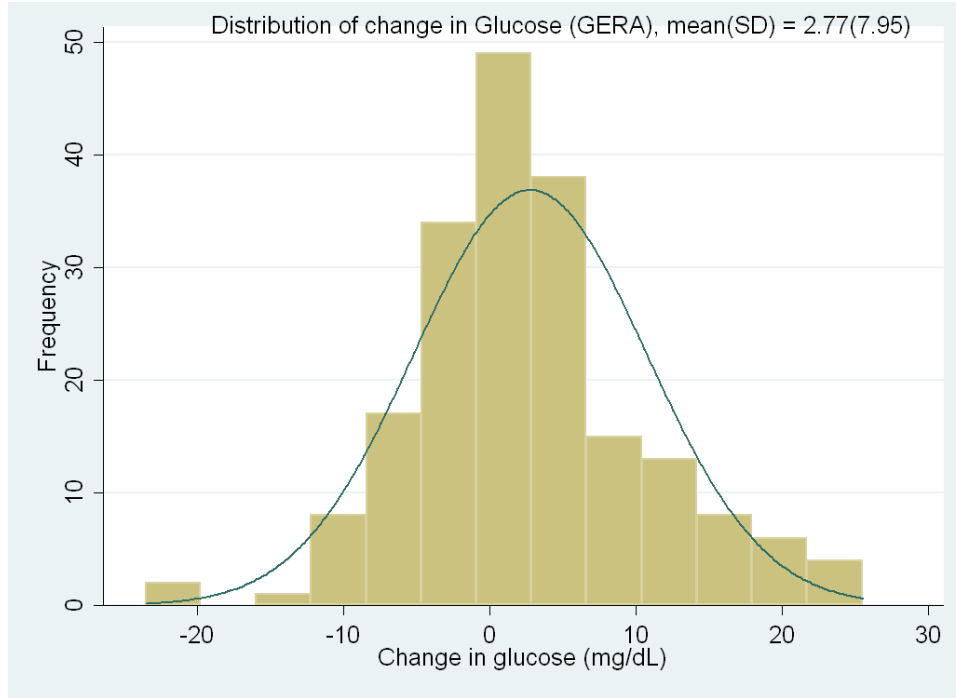
A

Variables	PEAR: Non-Hispanic Whites		p
	baseline	Post-treatment	
Total	229		
Gender(% men)	59.83		
Age(y)	49.97±9.4		
Body mass index(BMI)	30.33±4.9		
Plasma Glucose(mg/dL)	92.8±12.23	94.2±12.89	<.0001
Total Cholesterol(mg/dL)	196.8±34.68	204.02±38.09	<.0001
Plasma triglyceride(mg/dL)	149.55±100.9	160.92±122.64	0.03
Systolic blood pressure(mmHg)	151.8±13.14	141.42±14.43	<.0001
Diastolic blood pressure(mmHg)	97.9±5.8	93.2±8.6	<.0001

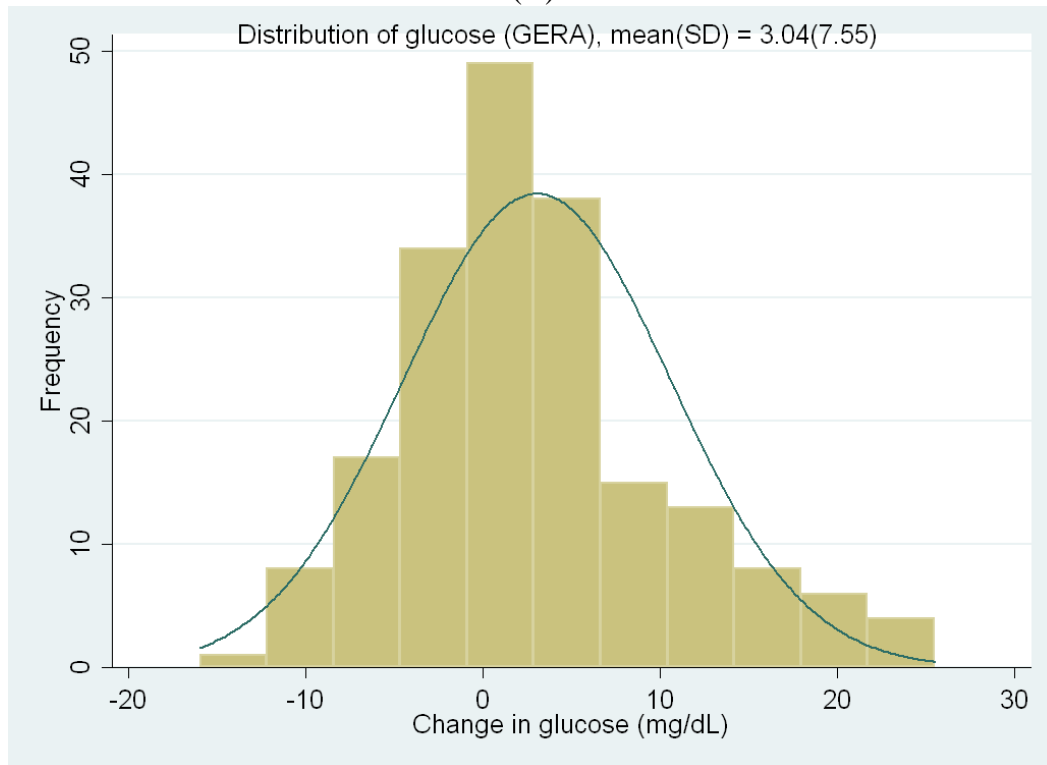
B

**Table 1:** (A) GERA sample characteristics measured at baseline and after hydrochlorothiazide treatment. (B) PEAR sample characteristics measured at baseline and after hydrochlorothiazide treatment

Figure 1 and Figure 2 show the distribution of inter-individual variation in plasma glucose response to HCTZ for GERA and PEAR respectively. Figure 1 A shows all of the available data, and Figure 1 B shows the distribution after removing individuals whose glucose response was beyond three standard deviations from the mean. The final GERA data consisted of 193 participants with mean and standard deviation for the change in plasma glucose of  $3.04 \pm 7.55$  and a skewness value of 0.697 and a kurtosis of 3.589. Figure 2 A presents the distribution of change in glucose from the PEAR study after removing participants with missing data (3 subjects). The distribution in Figure 2 B was generated after removing individuals over three standard deviations from the mean. These are the PEAR data values that were used in the analysis: 220 participants with a mean and standard deviation for the change in plasma glucose of  $1.44 \pm 8.94$ , and a skewness and kurtosis value of 0.007 and 3.328, respectively.



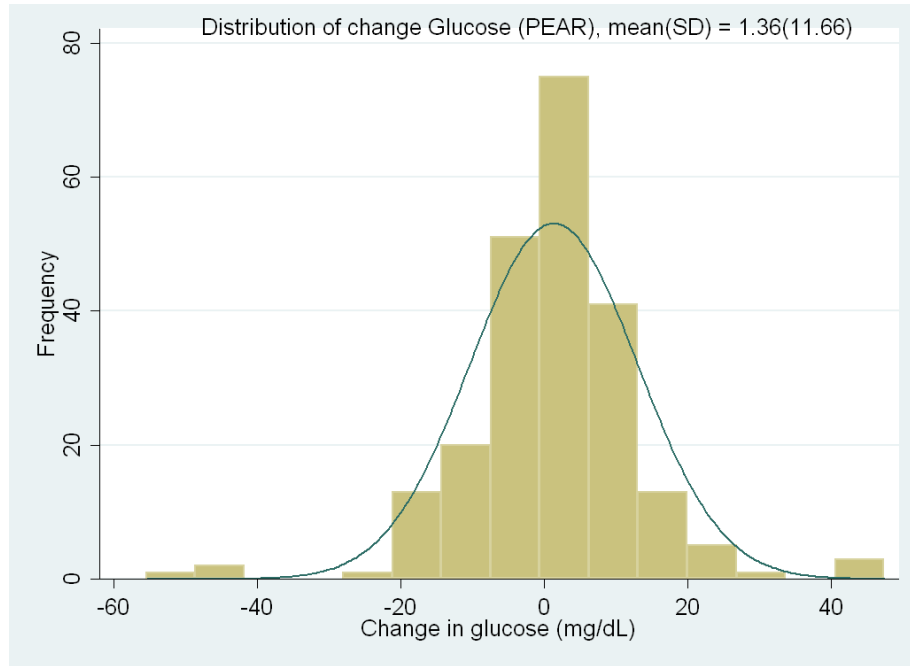
(A)



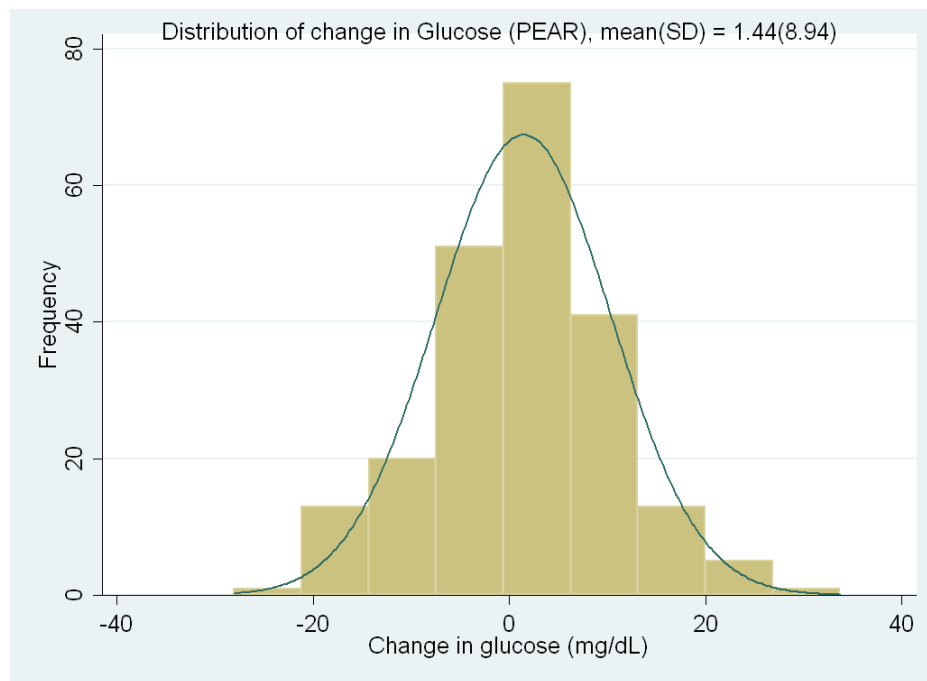
(B)

**Figure 1:** Interindividual variation of glucose response to hydrochlorothiazide in the GERA study.

(A) Glucose distribution before removing phenotypic values over three standard deviations from the mean. (B) Actual glucose distribution used in our GWAS analysis.



(A)



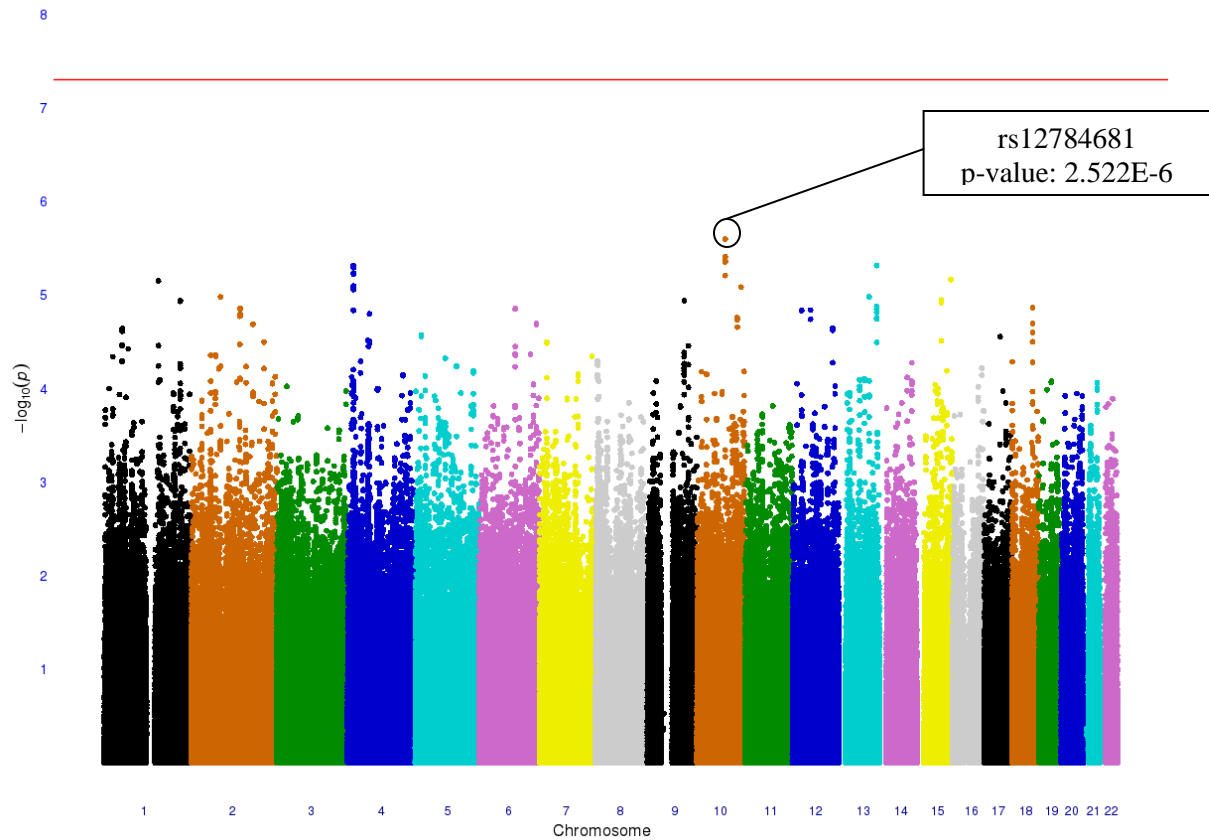
(B)

**Figure 2:** Interindividual variation of glucose response to hydrochlorothiazide in the PEAR study.

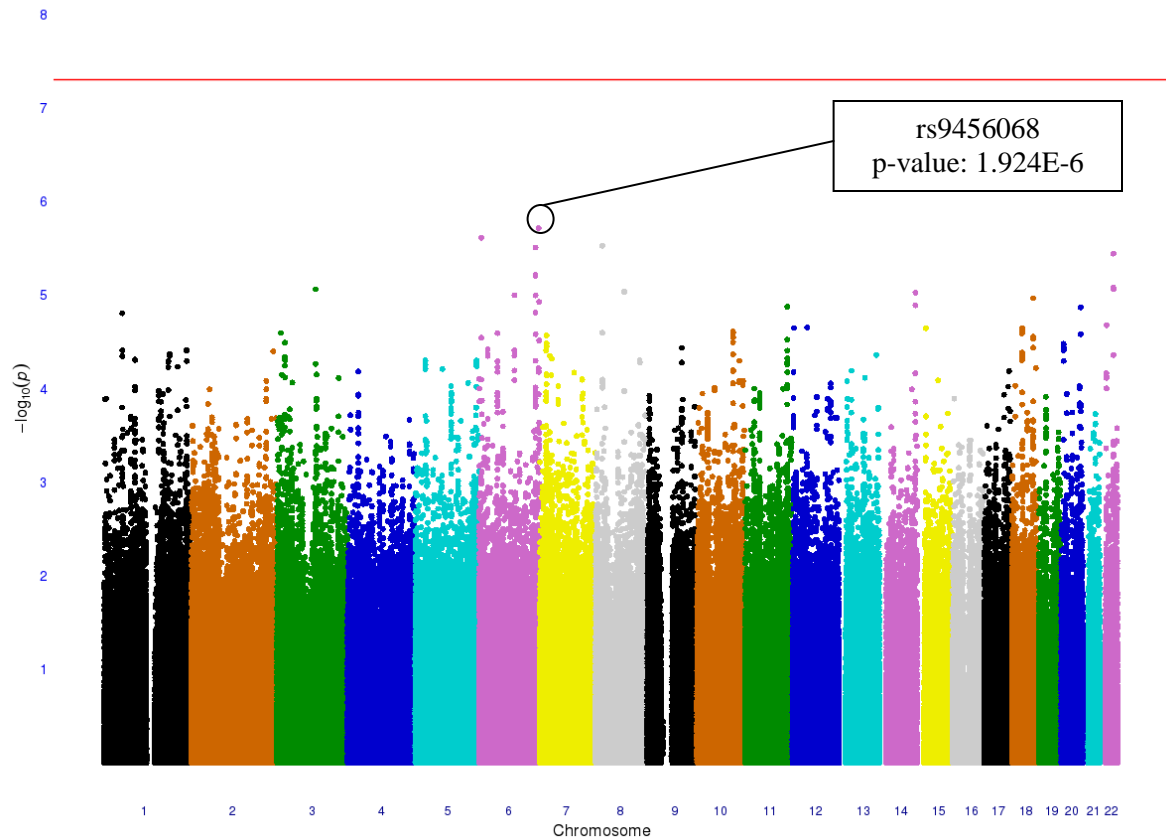
(A) Glucose distribution before removing phenotypic values three standard deviations from the mean. (B) Actual glucose distribution used in our GWAS analysis.

After quality control and imputation (see details in the Materials and Methods), there were a total of 2,124,464 SNPs to be analyzed in a sample of 193 non-Hispanics whites in GERA. In PEAR, the total number of SNPs was 2,182,834 in 220 non-Hispanics whites. The genome-wide association analysis results for GERA and PEAR are presented as Manhattan plots in Figure 3 and Figure 4, respectively. No single SNP reached the *a priori* genome-wide threshold of significance. The most significant SNPs were located on chromosome 10 at position 10q22-q23 (rs12784681 p-value: 2.522E-6) in GERA and on chromosome 6 at position 6q27 (rs9456068 p-value: 1.924E-6) in PEAR.



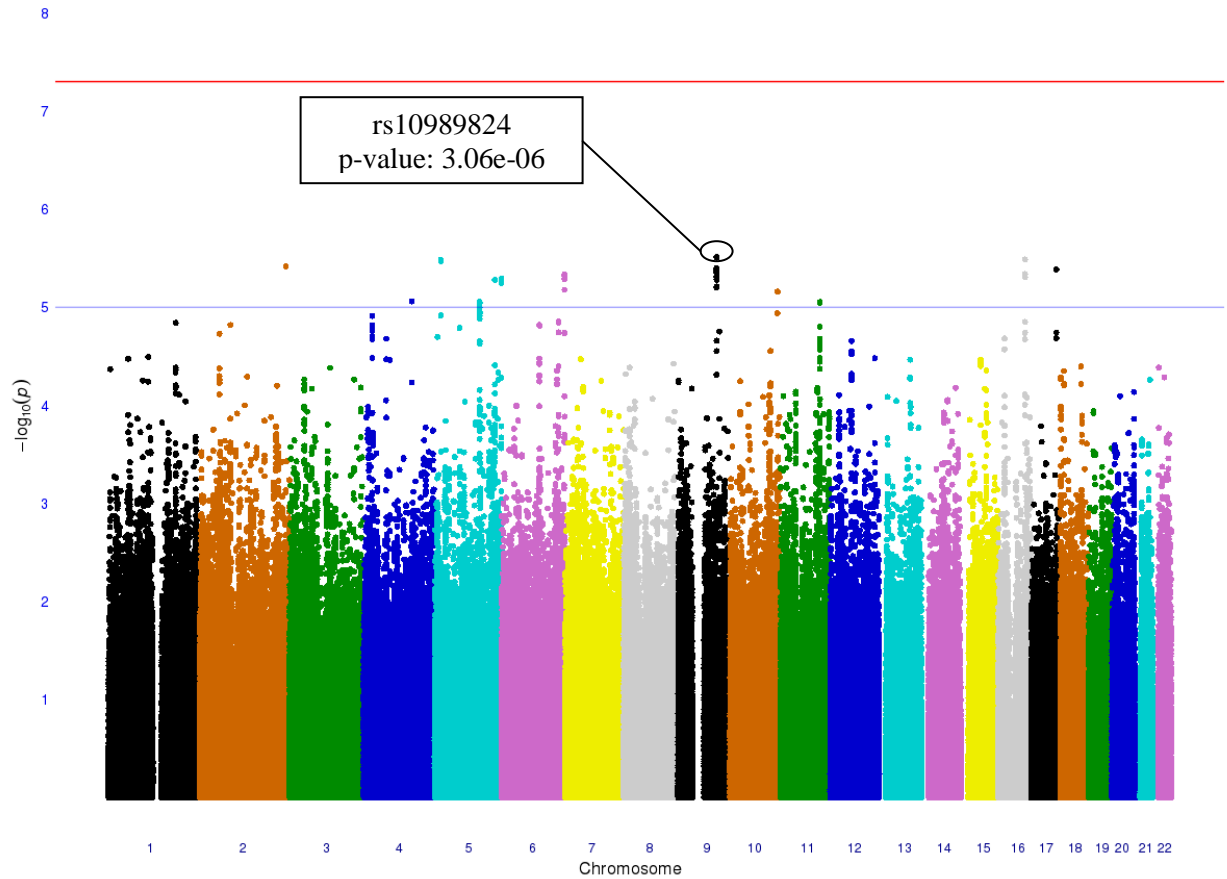


**Figure 3:** Manhattan plot of P values for SNP association to glucose response to hydrochlorothiazide in the GERA study. Observed p values versus chromosome location; the circle shows the top signal SNP rs12784681 ( $p=2.522 \times 10^{-6}$ ).



**Figure 4:** Manhattan plot of P values for SNP association to glucose response to hydrochlorothiazide in the PEAR study. Observed p values versus chromosome location; the circle shows the top signal SNP rs9456068 ( $p=1.924 \times 10^{-6}$ ).

Next, a fixed effects meta-analysis combining  $\beta$  estimates from the two GWAS scans (N=413 non-Hispanic whites, 2095294 SNPs) was carried out, and the results are shown in Figure 5. No single SNP reached the genome-wide significance threshold, but 50 SNPs in 9 regions (supplementary Table) reached our definition of suggestive significance ( $p\text{-value} \leq 1 \times 10^{-5}$ ) with the same direction of effects between GERA and PEAR).



**Figure 5:** Manhattan plot showing significant association of all SNP in the meta-analysis with glucose response to hydrochlorothiazide. Observed p values versus chromosome location; the circle shows the top signal SNP rs10989824 ( $p=3.06 \times 10^{-6}$ ).

Out of these 50 SNPs in the 9 genomic regions, 12 were chosen as representative of each region due to their lowest p-values and high LD with the other SNPs in the same region. These SNPs are shown in Table 2. The top SNP was located in region I, on chromosome 9. The top SNP, rs10989824 ( $p = 3.06 \times 10^{-6}$ ) has a  $\beta$  value indicating that with the presence of each minor allele (T) the change in glucose levels is increased by 5.98 mg/dL. Twenty-two other SNPs in high LD with rs10989824, are located in the same region (Supplementary Table 3 and Figure 6). The closest gene in this region is the Glutamate [NMDA] receptor subunit 3A (*GRIN3A*) which has been suggested to be involved in the development of synaptic elements by modulating NMDA receptor activity<sup>31</sup>

SNP	chr	minor alleles	MA F <sup>a</sup>	GERA			PEAR			Meta-analysis		
				beta	s.e.	Pvalue <sup>b</sup>	beta	s.e.	Pvalue <sup>b</sup>	beta	s.e.	Pvalue <sup>bc</sup>
rs10989824	9	T	0.06	-6.91	1.68	4.1e-5	-4.69	1.97	1.7e-2	-5.98	1.28	3.06e-6
rs427576	16	G	0.44	-2.99	0.78	1.4e-4	-2.25	0.82	5.9e-3	-2.63	0.57	3.25e-6
rs6870564	5	A	0.13	4.54	1.08	2.7e-5	2.87	1.29	2.6e-2	3.85	0.83	3.28e-6
rs1669070	2	T	0.40	-2.43	1.09	2.4e-2	-3.38	0.82	3.9e-5	-3.03	0.66	3.82e-6
rs11077614	17	G	0.49	-3.05	1.25	1.3e-2	-3.25	0.83	9.5e-5	-3.19	0.69	4.12e-6
rs7762018	6	A	0.13	2.39	1.17	3.8e-2	4.88	1.11	1.2e-5	3.70	0.81	4.59e-6
rs7702121	5	A	0.14	-3.59	1.16	1.8e-3	-3.98	1.18	7.6e-4	-3.78	1.28	5.09e-6
rs2114997	5	A	0.43	-2.12	0.75	4.0e-3	-2.93	0.81	2.8e-4	-2.49	0.55	5.25e-6
rs1551678	10	C	0.37	2.68	0.82	1.0e-3	2.66	0.86	1.9e-3	2.67	0.59	6.91e-6
rs1974942	4	A	0.11	-5.97	1.94	1.9e-3	-4.62	1.42	1.1e-3	-5.09	1.14	8.69e-6
rs890749	5	C	0.19	-2.74	1.01	6.1e-3	-3.70	1.03	3.4e-4	-3.21	0.72	8.81e-6
rs7130701	11	A	0.21	-3.08	0.93	8.5e-4	-3.02	1.02	3.1e-3	-3.05	0.67	8.84e-6

**Table 2:** SNPs showing association with glucose response to hydrochlorothiazide in meta-analysis. <sup>a</sup>Minor allele frequency among the combined sample of GERA and PEAR. <sup>b</sup>P values are based on a linear regression adjusted for sex, age, BMI, baseline glucose and the two first principal components of genetic variation for each study. <sup>c</sup>P values are calculated using inverse-variance meta-analysis.

Region II, on chromosome 16 contains the second highest SNP with suggestive associations with changes in glucose due to HCTZ therapy. The SNP rs427576 (pvalue= $3.25 \times 10^{-6}$ ) along with 2 other SNPs were located in a genomic region without well-annotated genes as shown in Figure 7. The SNP rs6870564 (pvalue= $3.28 \times 10^{-6}$ ) in region III-A, on chromosome 5 (Figure 8) is the third most significant region associated with change in glucose levels in response to HCTZ therapy. The region contains the *MYO10* which is related to the myosin heavy chain (MCH) genes. This gene was linked to the pathogenesis of type II diabetes<sup>32</sup>.

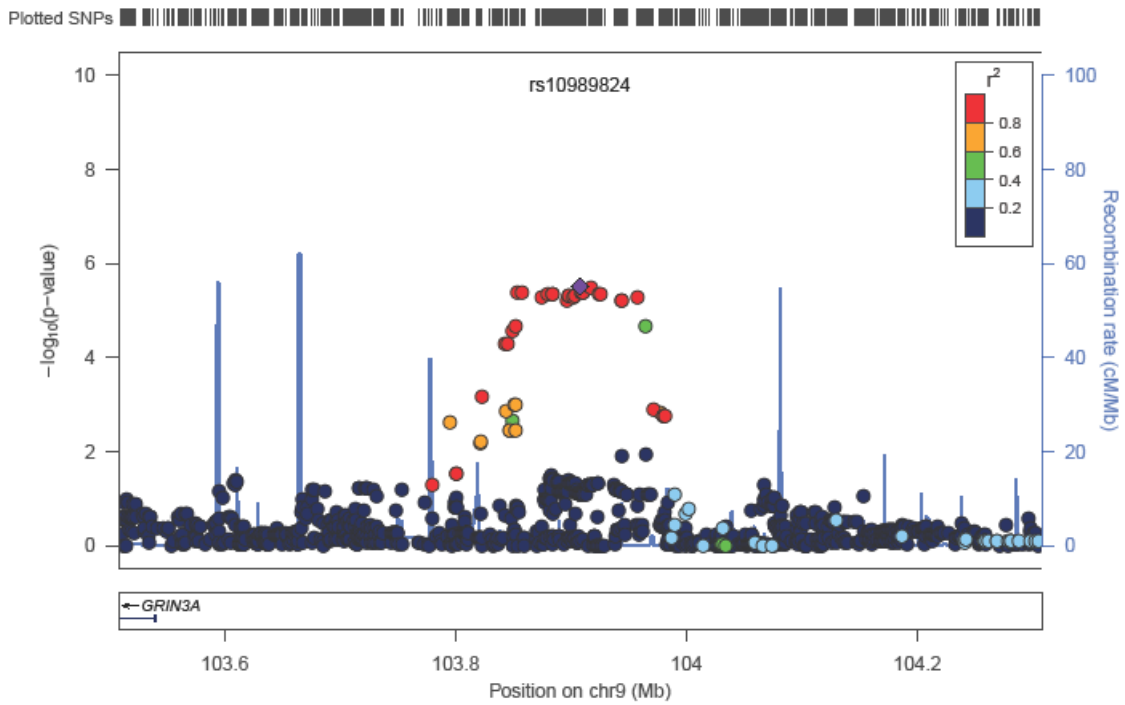
The sixth most significantly associated SNP rs7762018 (pvalue= $4.59 \times 10^{-6}$ ), was in genomic region VI on chromosome 6, along with 4 other SNPs (rs6923339, rs6941766, rs6904601, rs4716942, rs1127489). This chromosomal location, 6q27 (Figure 9), was previously reported in a Genome-Wide Meta-Analysis as associated with Type I Diabetes<sup>33</sup>. In region III-B (Figure 10), the *MGAT1* gene, which was close to the SNP rs7702121 (pvalue= $5.09 \times 10^{-6}$ ), was associated with BMI, body weight and fatty acid metabolism<sup>34, 35</sup>.

Figure 11 and Figure 12 show regional plots of the rest of the genomic regions: region III-C and III-D were located on chromosome 5, region IV on chromosome 2, region V on chromosome 17, region VII on chromosome 10, region VIII on chromosome 4, and region IX on chromosome 11. Genes found in these regions are listed in Table 3

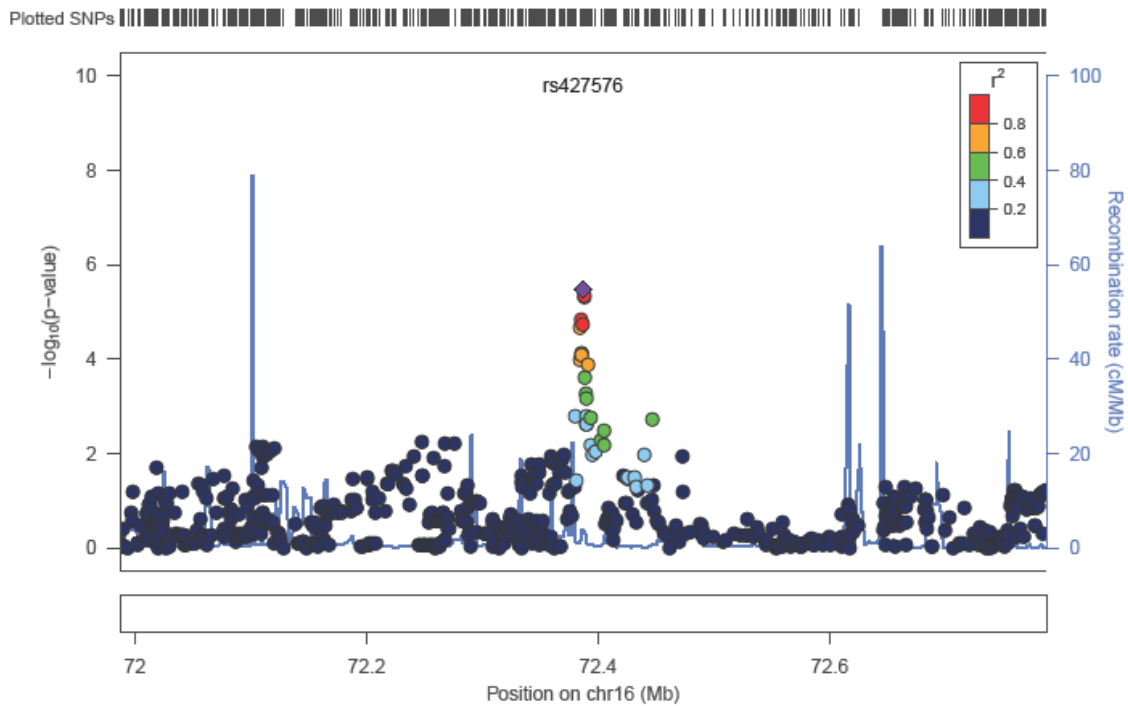
Gene	Symbol
Ferritin mitochondrial	<i>FTMT</i>
Serum response factor binding protein 1	<i>SRFBP1</i>
Lysyl oxidase	<i>LOX</i>
Zinc finger protein 474	<i>ZNF474</i>
Synuclein- alpha interacting protein	<i>SNCAIP</i>
Cyclin	<i>G1CCNG1</i>
NudC domain containing 2	<i>NUDCD2</i>
Hyaluronan-mediated motility receptor	<i>RHAMM</i>
Methionine adenosyltransferase II beta	<i>MAT2B</i>
Neuromedin U receptor 1	<i>NMUR1</i>
5-hydroxytryptamine (serotonin) receptor	<i>2BHTR2B</i>
Solute carrier family 39 (metal ion transporter)- member 11	<i>SLC39A11</i>
Sodium channel and clathrin linker 1	<i>SCLT1</i>
Guanylate cyclase 1, soluble, alpha 2	<i>GUCY1A2</i>
CWF19-like 2, cell cycle control (S. pombe)	<i>CWF19L2</i>
AlkB, alkylation repair homolog 8 (E. coli)	<i>ALKBH8</i>
ELMO/CED-12 domain containing 1	<i>ELMOD1</i>

**Table 3:** List of genes found other suggestive regions after Meta-analysis

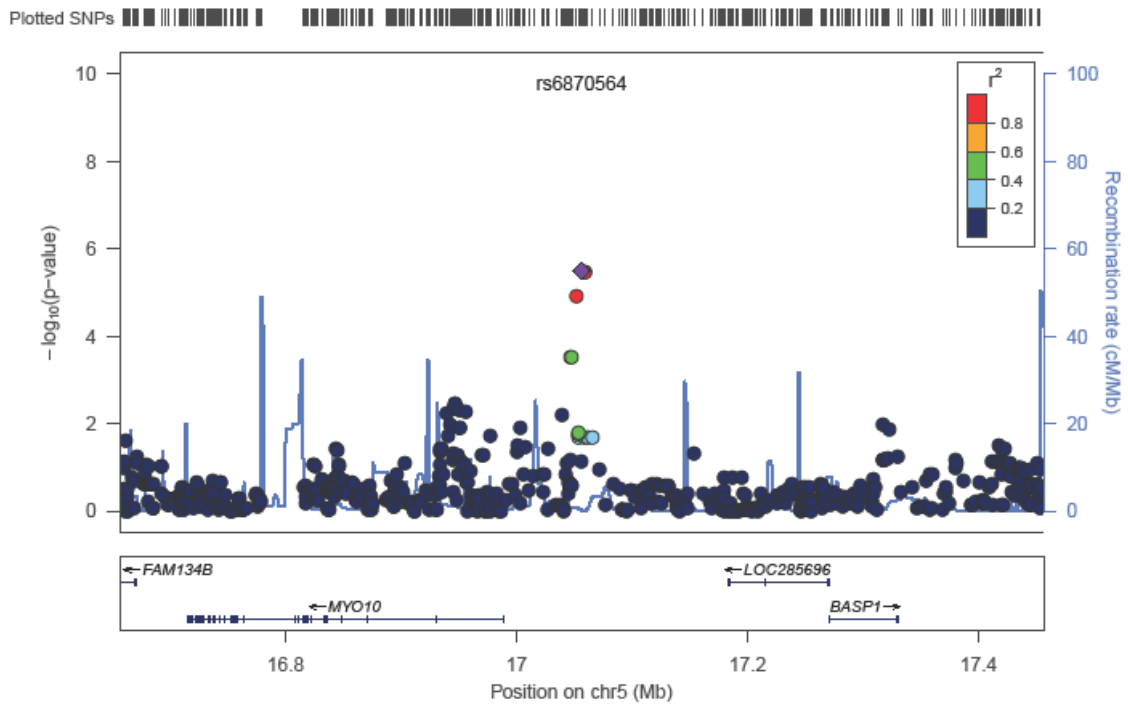




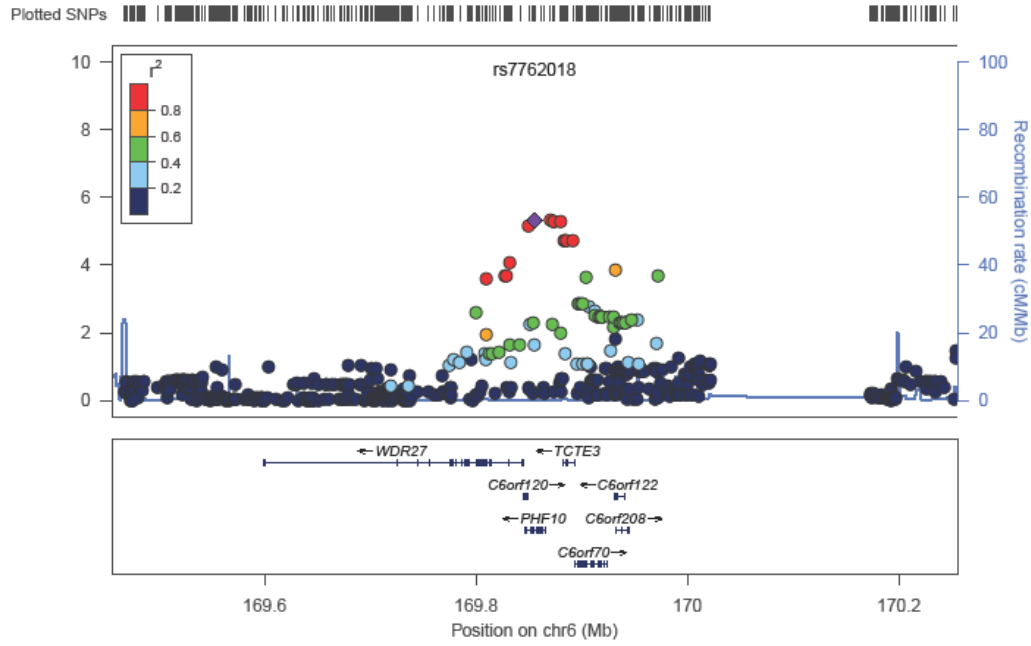
**Figure 6:** Regional plot of the most significant SNP on chromosome 9. The top SNP is represented by a purple diamond. The  $-\log_{10}$  p-values are on the left y-axis and the right y-axis shows the estimates rates of recombination. The SNP positions are on the x-axis, as well as the genes which flank a 400kb region. The gene *GRIN3A* was the closest to the region associated with change in glucose due to HCTZ



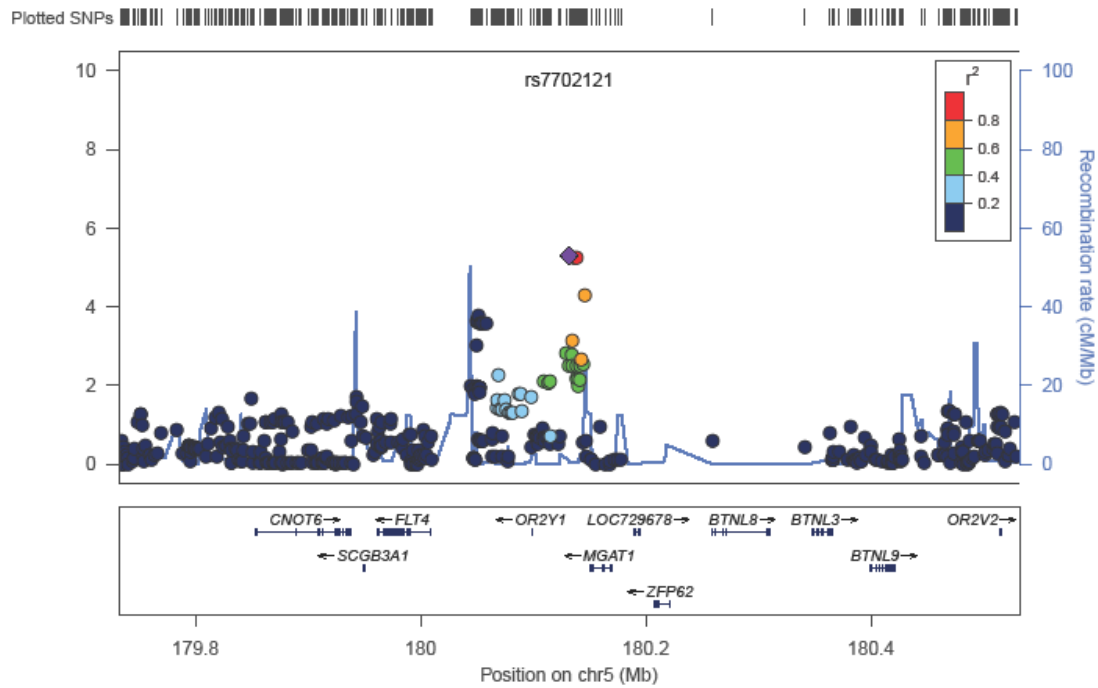
**Figure 7:** Regional plot of the second SNP associated to change in glucose due to HCTZ. The SNP rs427576 on chromosome 16 is located in a gene desert. The top SNP is represented by a purple diamond. The  $-\log_{10}$  p-values are on the left y-axis meanwhile the right y-axis shows the estimates rates of recombination. The SNP positions are on the x-axis as well as the genes which flank a 400kb region



**Figure 8:** Regional plot on chromosome 5. The *MYO10* gene, which is located in the region, is related to the myosin heavy chain (MHC) gene family. These genes were linked to T2D<sup>32</sup>. The top SNP is represented by a purple diamond. The  $-\log_{10}$  p-values are on the left y-axis and the right y-axis shows the estimates rates of recombination. The SNP positions are on the x-axis as well as the genes which flank a 400kb region



**Figure 9:** Regional plot of fourth most significant SNP on chromosome 6. This chromosomal location, 6q27 was previously reported in a Genome-Wide Meta-Analysis as associated with T1D<sup>33</sup>. The top SNP is represented by a purple diamond. The  $-\log_{10}$  p-values are on the left y-axis meanwhile the right y-axis shows the estimates rates of recombination. The SNP positions are on the x-axis as well as the genes which flank a 400kb region



**Figure 10:** Regional plot of chromosome 5 showing *MGAT1* gene which has been previously associated with BMI, body weight and fatty acid metabolism<sup>34, 35</sup>. The top SNP is represented by a purple diamond. The  $-\log_{10}$  p-values are on the left y-axis and the right y-axis shows the estimates rates of recombination. The SNP positions are on the x-axis as well as the genes which flank a 400kb region

## Discussion

In this study, we performed a meta-analysis of GWAS result from two non-Hispanic white pharmacogenetic studies consisting in total of over 425 individuals. We were not able to identify any locus which met the *a priori* defined genome-wide significance level for a GWAS for change in glucose level in response to HCZT treatment. However 50 SNPs in 9 regions reached our criteria of suggestive association, and three of these regions were previously reported to be associated with relevant phenotypes in different GWA studies.

HCTZ use has been suggested to be related to hyperglycemia in many clinical trials and observational studies, as well as a recent meta-analysis in comparison to other antihypertensive drugs<sup>19</sup>. I conclude that there are unlikely to be common SNPs with large effects on the adverse metabolic effects to hydrochlorothiazide treatment, and that large sample sizes will be needed for pharmacogenetic studies of inter-individual variation in response commonly prescribed medication.

We observed suggestive evidence of association with change in glucose due to HCTZ for rs7702121. How the SNP rs7702121 may contribute to the change in glucose is unknown and the likelihood of this SNP being the causal variant is low. However this SNP is in high LD with the coding exon in *MGAT1* which has been associated with body weight, BMI and fatty acid metabolism<sup>34</sup>. Mannosyl(alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (*MGAT1*) is an enzyme related to the synthesis of protein and lipids<sup>36</sup>. *MGAT1* is a major contributor in the Monoacylglycerol pathway which produces Diacylglycerol (DAG)<sup>37</sup>. Elevated levels of DAG and increased activity of protein kinases C (PKC) are found in different tissues in diabetic individuals<sup>38</sup>. Miele et al<sup>39</sup> suggested that DAG regulates an important pathway in which glucose promotes the degradation of DAG as

well as translocation of PKC from the plasma membrane to the cytoplasm. Removal of PKC activates the insulin receptor cascade leading to GLUT4 membrane translocation and glucose uptake<sup>39</sup>. Therefore I hypothesize that HCTZ modifies the activity of *MGAT1* which will increase the production of DAG; avoiding, in this way, the capture of glucose by *GLUT4* leading to an increase of plasma glucose in hypertensive individuals taking a diuretic.

In another region, on chromosome 5, the SNP rs6870564 also showed suggestive evidence of association with change of in plasma glucose. This SNP is in close proximity to the *MYO10* gene which codes an unconventional myosin<sup>40</sup> that participates in different cellular processes such as membrane trafficking to signaling and cell motility. *MYO10* may have a relationship with the pathogenesis of T2D as suggested by Olsson et al<sup>32</sup>. Contraction is a stimulant for the translocation of *GLUT4* in skeletal muscle<sup>32, 41</sup>. Alteration in the expression of myosin in response to HCTZ may prevent the movement of *GLUT4* to the plasma membrane of these cells which, in turn, would impede uptake of glucose into the cell and accumulation of glucose in blood.

The last region to be discussed is on chromosome 6 where the SNP rs7762018 reaches suggestive evidence of association to change in glucose due to HCTZ. The SNP was located in a 6q27 region which has been related to T1D in a genome-wide meta-analysis<sup>33</sup> and other autoimmune diseases<sup>42</sup>.

It has been hypothesized that the mechanism by which HCTZ leads to hyperglycemia involves increased potassium excretion and hypokalemia. It is interesting to note, therefore, that none of the genes identified to be suggestively associated with change in glucose were obviously related potassium transport or excretion<sup>16</sup>. Therefore, it is more

likely that the mechanism involves a direct link with insulin sensitivity and metabolism as suggested by Eriksson et al.<sup>22</sup>

In summary, the primary objective of this thesis was to identify genes or chromosomal regions that show an association with change in glucose levels in response to HCTZ in non-Hispanics whites. Although we were not able to reach GWAS significant p-values, 50 SNP in 9 regions had suggestive p-values. Large sample sizes and further meta-analyses will be necessary to identify contributing to inter-individual variation in glucose response to HCTZ and likely other pharmacologic phenotypes.

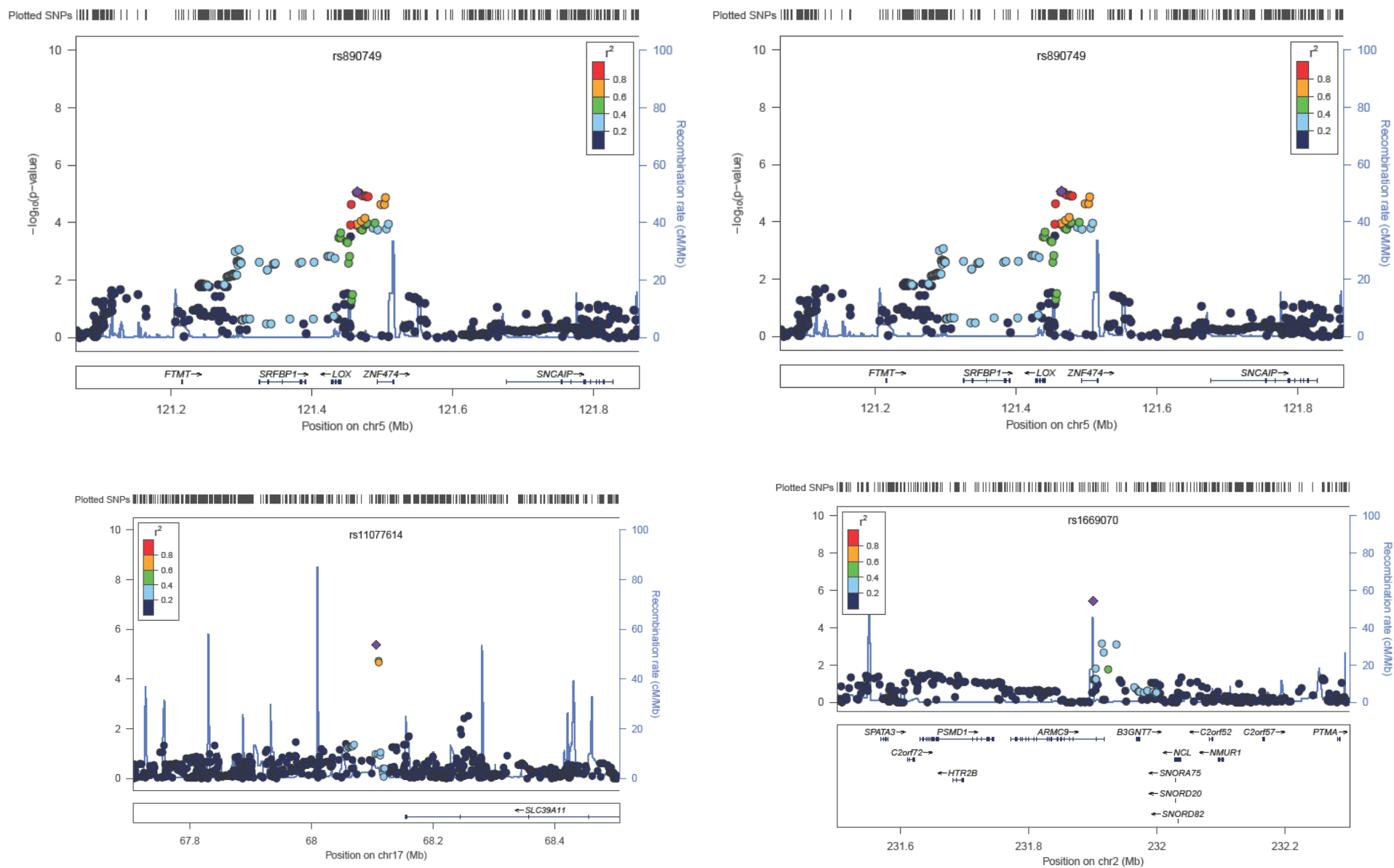


## APPENDIX

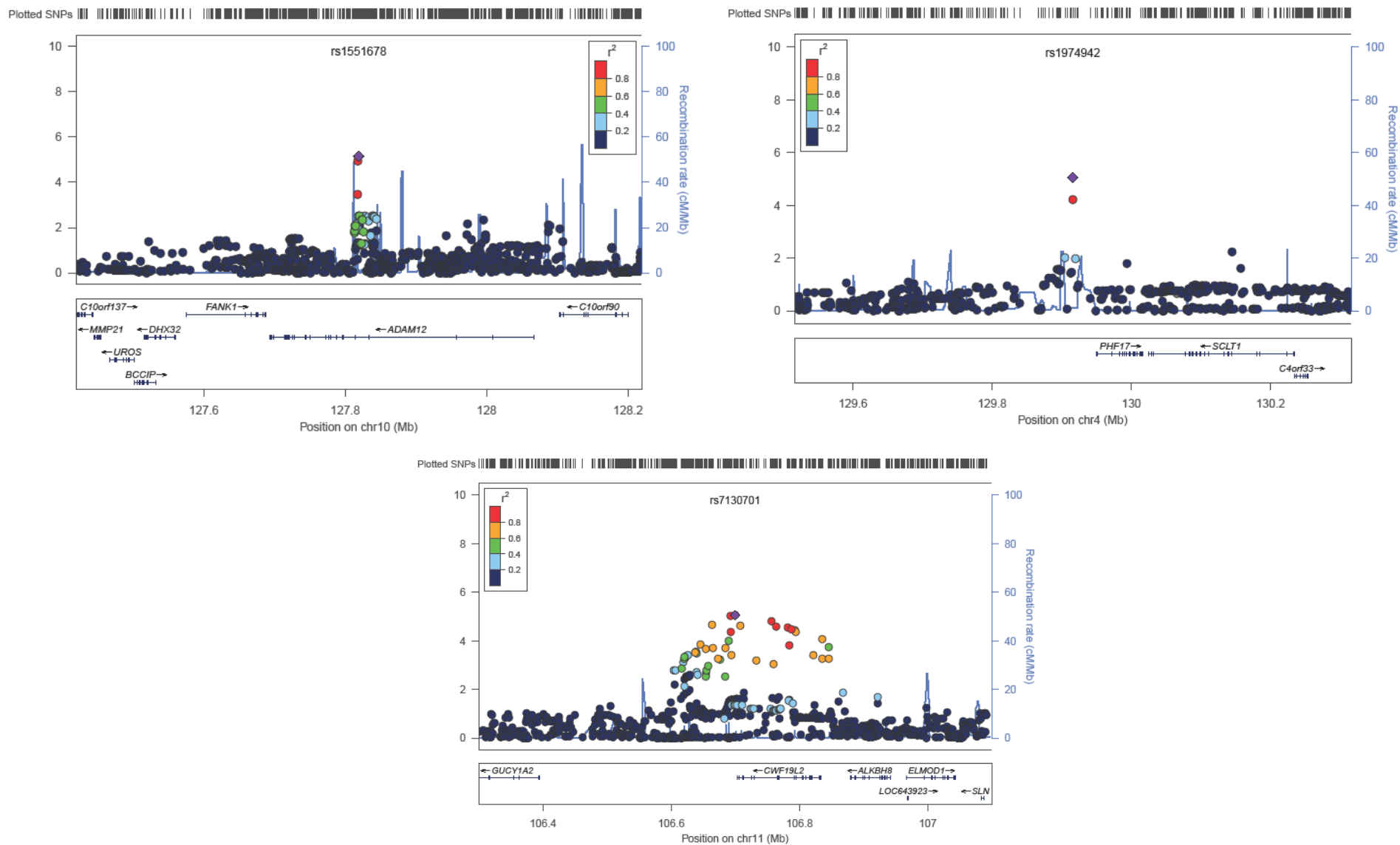
Region		Name	chr	Allele1	Allele2	Position	Freq Allele1	Direction	$\beta$	P.value
Region I		rs10989824	9	T	C	103907659	0.0599	--	-5.980	3.06E-06
		rs7034610	9	A	G	103917056	0.0614	++	5.856	3.11E-06
		rs10820064	9	A	T	103909080	0.0674	++	5.7556	3.99E-06
		rs10989829	9	A	C	103909525	0.0674	++	5.7506	4.01E-06
		rs10989833	9	T	G	103909860	0.0675	--	-5.743	4.02E-06
		rs10989837	9	T	C	103910291	0.9324	++	5.731	4.07E-06
		rs10989791	9	A	G	103857420	0.0592	++	5.8256	4.21E-06
		rs7020246	9	T	G	103852964	0.9407	++	5.824	4.25E-06
		rs10760837	9	T	C	103879660	0.059	--	-5.862	4.31E-06
		rs10760841	9	A	G	103924080	0.939	--	-5.749	4.37E-06
		rs16921277	9	A	G	103925122	0.939	--	-5.749	4.37E-06
		rs1449650	9	T	G	103883859	0.0589	--	-5.860	4.45E-06
		rs10820051	9	T	G	103884041	0.9411	++	5.86	4.47E-06
		rs10820055	9	T	C	103897774	0.0589	--	-5.847	4.79E-06
		rs895989	9	T	C	103898526	0.0588	--	-5.845	4.82E-06
		rs10760840	9	A	G	103903001	0.0659	++	5.7746	4.92E-06
		rs10989820	9	T	C	103901773	0.0588	--	-5.837	5.00E-06
		rs10820046	9	A	G	103874453	0.9343	--	-5.722	5.24E-06
		rs1597340	9	T	G	103957354	0.0616	--	-5.686	5.24E-06
		rs1449663	9	A	G	103896314	0.9345	--	-5.698	6.13E-06
		rs10820076	9	T	C	103943684	0.9323	++	5.570	6.23E-06
		rs10760846	9	T	C	103944275	0.9323	++	5.567	6.28E-06
Region II		rs427576	16	A	C	72386771	0.5617	--	-2.635	3.25E-06
		rs417278	16	T	C	72387977	0.5811	++	2.622	4.58E-06
		rs379401	16	C	G	72387865	0.587	--	-2.608	4.92E-06
Region III	A	rs6870564	5	A	G	17056085	0.1287	++	3.8521	3.28E-06
		rs11953330	5	T	C	17059372	0.8716	++	3.846	3.39E-06
		rs11959796	5	A	T	17059405	0.1285	++	3.8473	3.39E-06
	B	rs7702121	5	A	C	180131859	0.143	--	-3.783	5.09E-06
		rs6859974	5	T	C	180138544	0.1436	++	3.731	5.63E-06
		rs1021001	5	C	G	180136710	0.1435	--	-3.734	5.67E-06
	C	rs890749	5	T	C	121464495	0.809	--	-3.211	8.81E-06
		rs1834915	5	A	T	121465418	0.1911	--	-3.210	8.82E-06
		rs2560322	5	C	G	121467234	0.8087	++	3.2076	8.89E-06
		rs890750	5	T	G	121463478	0.8098	--	-3.209	9.01E-06
		rs970041	5	A	C	121467568	0.1924	--	-3.173	1.00E-05

	D	rs2114997	5	A	G	162584192	0.4274	--	-2.494	5.25E-06
<b>Region IV</b>		rs1669070	2	T	C	231900549	0.4029	--	-3.032	3.82E-06
<b>Region V</b>		rs11077614	17	A	G	68106224	0.5048	--	-3.188	4.12E-06
<b>Region VI</b>		rs7762018	6	A	C	169855169	0.1319	++	3.703	4.59E-06
		rs6923339	6	A	T	169869958	0.132	++	3.6979	4.73E-06
		rs6941766	6	A	G	169873578	0.8679	--	-3.685	5.10E-06
		rs6904601	6	T	G	169873830	0.8679	++	3.684	5.11E-06
		rs4716342	6	A	G	169879884	0.8679	--	-3.683	5.15E-06
		rs1127489	6	T	C	169849627	0.1292	--	-3.647	6.62E-06
<b>Region VII</b>		rs1551678	10	C	G	127819002	0.3698	++	2.6746	6.91E-06
<b>Region VIII</b>		rs1974942	4	A	G	129915671	0.1052	--	-5.089	8.69E-06
<b>Region IX</b>		rs7130701	11	A	G	106699449	0.7894	--	-3.048	8.84E-06
		rs12274982	11	T	C	106699749	0.2106	--	-3.047	8.89E-06
		rs4754214	11	A	C	106692106	0.7894	--	-3.048	8.96E-06

**Table 4-Supplementary data:** Top 50 SNPs associated with change of Glucose levels in Non-Hispanics white patients. These SNP were located in 9 genomic regions. In the case of chromosome 5, four different sub-regions were identified.



**Figure 11:** Regional plots of 4 loci (chromosome 5, 17 and 2). These loci show suggestive association ( $p\text{-values} < 1 \times 10^{-5}$ ) with change in glucose due to HCTZ. The top SNP is represented by a purple diamond.  $-\log_{10} p\text{-values}$  are on the left y-axis meanwhile the right y-axis shows the estimates rates of recombination. The SNP positions are on the x-axis as well as the genes which flank a 400kb region



**Figure 12:** Regional plots of 3 loci (chromosome 10, 4 and 11). These loci show suggestive association ( $p$ -values  $< 1 \times 10^{-5}$ ) with change in glucose due to HCTZ. The top SNP is represented by a purple diamond.  $-\log_{10}$   $p$ -values are on the left y-axis meanwhile the right y-axis shows the estimates rates of recombination. The SNP positions are on the x-axis as well as the genes which flank 400kb region

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